Clinical Study

ONCOLOGY

Oncology 1998;55:33–38

The Prognostic Significance of c-erbB-2 Serum Protein in Metastatic Breast Cancer

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Key Words
Metastatic breast cancer
c-erbB-2
Prognosis
Survival

Abstract
The relationship between c-erbB-2 serum positivity and prognosis was evaluated in 80 patients with metastatic breast cancer. Using 120 fmol/ml as a cutoff level, elevated concentrations were found in 31 patients (38.8%) at the time of detection of metastases. Menopausal status, steroid receptor status, site of recurrence, initial tumor size, initial degree of nodal involvement as well as relapse-free interval were unrelated to c-erbB-2 serum positivity. In addition, no association could be found between adjuvant chemotherapy and positive c-erbB-2 concentrations. Patients with elevated c-erbB-2 levels showed a lower response rate (including complete remission, partial remission, no change) to first-line therapy than those with normal levels (29 vs. 59%, $p < 0.01$). The median survival time after relapse was 12 months (CI: 3–22 months) for the c-erbB-2-negative patients and only 6 months (CI: 3–8 months) for the c-erbB-2-positive group ($p < 0.01$). In the multivariate analysis, while c-erbB-2 levels at the time of primary surgery had no significant impact on survival in metastatic breast cancer, serum c-erbB-2 turned out to be the strongest factor for predicting survival after relapse.

Introduction

During the past few years, evidence has increased that alterations of proto-oncogenes contribute to the pathogenesis of cancer. An intensively studied proto-oncogene in human breast cancer is c-erbB-2 located on chromosome 17q21. It encodes a 185-kD surface glycoprotein that has extensive homology to the EGF receptor [1, 2]. Gene amplification or overexpression of c-erbB-2 gene product occurs in 15–30% of breast carcinomas, and in several studies was shown to be associated with poor prognosis in breast cancer patients [3–7].

The external domain of c-erbB-2 protein is frequently secreted and can be detected in human serum [8–11]. Elevated c-erbB-2 concentrations have been reported in approximately 5–30% of primary breast cancer patients. Recent investigations indicate that c-erbB-2 may be a useful prognostic serum marker in primary breast cancer [12–15]. This study was undertaken to evaluate the prognostic significance of c-erbB-2 serum positivity in predicting survival after relapse.

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0030–2414/98/0551–0033$15.00/0

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Materials and Methods

Patients

Patients were drawn from a retrospective case-controlled study (n = 211) which evaluated the influence of serum c-erbB-2 on response to adjuvant chemotherapy in node-positive breast cancer (> 3 nodes positive). Patients had received CMF, NC or FNC as adjuvant chemotherapy regimen. From this investigation, patients who developed metastases were included in the present study. Serum samples at the time of detection of metastases were available from 80 of the 112 recurrent breast cancer patients. Twenty-three of these patients received hormonal therapy as first-line treatment, 57 underwent cytotoxic chemotherapy. Clinical response to first-line therapy was evaluated according to the criteria of UICC (International Union against Cancer).

Clinical characteristics of these patients are shown in table 1. For the diagnosis of metastases, abdominal sonography, chest X-ray and bone scintigraphy had been performed. Hormone receptor status was determined in tumor tissue using a dextran-coated charcoal ligand binding assay at the time of primary breast cancer. Estrogen and progesterone receptor levels ≧ 10 fmol/mg were considered positive. The median follow-up of the metastatic breast cancer patients was 8 months (range: 1–78 months).

C-erbB-2 Measurement

Serum samples were collected at the time of initial diagnosis of metastases and remained in a blood bank at –20°C until assayed. c-erbB-2 protein was measured in serum by an automated chemiluminescent immunoassay. Briefly, serum samples (25 µl), in triplicate, were incubated at 37°C with monoclonal antibodies Tab-264 (capture MAb conjugated to paramagnetic particles) and Tab-259 (label Mab conjugated to acridinium ester). After a 7.5-min incubation the particles were washed twice. The ester was then activated and the chemiluminescent signal was measured by a luminometer. The signal was proportional to the amount of c-erbB-2 protein in serum. This immunoassay, kindly provided by Ciba Corning Diagnostics, a Chiron business, Alameda, Calif., USA, correlates with the commercially available Triton Laboratories' c-erbB-2 serum enzyme immunoassay kit. For this study 1 U/ml of the Triton kit equals 5 fmol/ml of the chemiluminescent assay. The intra- and interassay coefficients of variation were less than 5%. Based on measurements in 63 apparently healthy females, the cutoff level of c-erbB-2 was defined at 120 fmol/ml [15]. Preoperative c-erbB-2 levels were available from 79 of the 80 patients who developed recurrent disease and c-erbB-2 concentrations were also determined for these samples.

Statistics

c-erbB-2 was analyzed as a dichotomous variable (c-erbB-2-'positive' versus c-erbB-2-'negative'). Correlations between variables were evaluated by the chi-squared test. Relapse-free interval was calculated from the time of diagnosis of primary breast cancer until the date of recurrence. Survival after relapse was defined as the time interval between first diagnosis of recurrence and death. Survival curves were generated using the methods of Kaplan-Meier and were compared by the log-rank test. A Cox regression model was performed in the univariate analysis to screen for prognostic factors and in the multivariate analysis to determine their relative importance in predicting survival after relapse. The factors included in the survival analysis were menopausal status, initial tumor size, initial degree of nodal involvement, steroid receptor status, relapse-free interval, c-erbB-2 levels (at the time of primary surgery and at the time of diagnosis of metastatic disease), adjuvant chemotherapy as well as site of recurrence. Odds ratios (OR) and their 95% confidence intervals (CI) were also calculated by the Cox regression model.

Table 1. Characteristics of the 80 metastatic breast cancer patients

Variable	Patients
Menopausal status	
Premenopausal	43
Postmenopausal	37
Initial tumor size	
pT1	13
pT2–4	62
Unknown	5
Initial degree of nodal involvement	
4–9 nodes positive	30
>9 nodes positive	50
Steroid receptor status	
ER-positive	31
PR-positive	50
Adjuvant chemotherapy	
CMF	24
FNC	12
NC	44
C-erB-2 at primary surgery	
Positive	12
Negative	67
Unknown	1
Site of recurrence	
Bone	29
Visceral site[1]	50
Unknown	1
Relapse-free interval	
≤ 1 year	31
>1 year	49

ER = Estrogen receptor; PR = progesterone receptor.

[1] Including lung (n = 23), liver (n = 16), brain (n = 5), abdomen (n = 3), multiple (n = 3).

Results

Serum c-erbB-2 concentrations of the 80 metastatic breast cancer patients ranged from 31.6 to 7,740.0 fmol/ml with a median of 103.0 fmol/ml (mean 445.4 9 fmol/ml). Using 120 fmol/ml as cutoff level, 31 patients (38.8%) had elevated c-erbB-2 values.

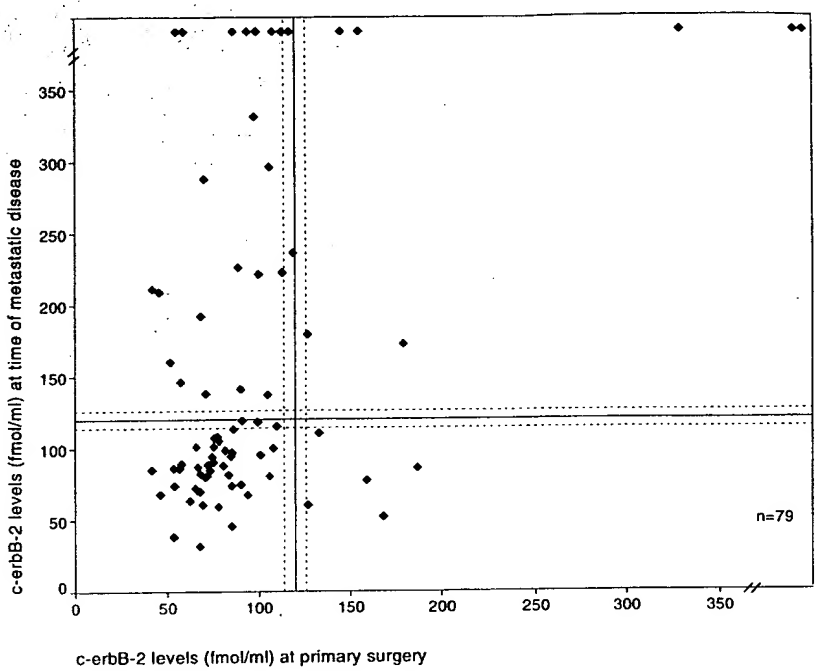

Fig. 1. C-erbB-2 levels at the time of detection of metastatic disease related to c-erbB-2 levels at primary surgery. The cutoff level (= 120 fmol/ml) is indicated by the solid line (--- 120 fmol/ml ± 5%, interassay coefficient).

Menopausal status (p = 0.3), initial tumor size (p = 0.9), initial degree of nodal involvement (p = 0.9), estrogen receptor (p = 0.2) and progesterone receptor status (p = 0.9) did not correlate with c-erbB-2 concentrations. The relapse-free interval was also unrelated to c-erbB-2 values (p = 0.2). No relationship between any specific site of distant metastases and elevated c-erbB-2 concentrations could be detected (p = 0.5). However, the highest c-erbB-2 values were generally found in patients with bone metastases. There was no correlation between type of adjuvant treatment chosen for patients at primary surgery and c-erbB-2 serum positivity (p = 0.6).

c-erbB-2 levels were available from 79 of the 80 patients at the time of primary surgery. Twelve (15.2%) of the 79 metastatic patients had positive c-erbB-2 values preoperatively, while 67 (84.8%) patients were c-erbB-2-negative. In the prior case-controlled study, only 7.1% (7/99) of patients without recurrence had elevated c-erbB-2 levels [15].

A change of c-erbB-2 serum positivity during the clinical course could be detected in nearly 35% of these recurrent patients (fig. 1). Twenty-three of the 67 (34.3%) c-erbB-2-negative patients had positive c-erbB-2 values at the time of detection of metastases. Conversely, 5 of the 12 (41.7%) positive patients had a decrease in c-erbB-2 concentrations to normal. Four of these patients metastasized to the bone and one to the lung.

Table 2 presents the correlation between c-erbB-2 serum positivity and response to treatment. Unrelated to type of first-line therapy (chemotherapy or endocrine therapy) c-erbB-2-positive patients were less likely to benefit from treatment compared to c-erbB-2-negative ones. The response rate (complete remission + partial remission + no change) of patients with nonelevated c-erbB-2 levels was 59% (29/49 patients), while only 29% (9/31 patients) of the c-erbB-2-positive patients had no disease progression ($p < 0.01$).

The univariate analyses demonstrated that elevated c-erbB-2 levels at the time of detection of metastases were associated with a shorter survival after relapse (table 3). The median survival time for the c-erbB-2-positive patients was 6 months (CI: 3–8 months). This was significantly shorter compared to 12 months (CI: 3–22 months) for the c-erbB-2-negative group ($p < 0.01$). Figure 2 shows the survival curves after relapse according to c-erbB-2 positivity. Patients with elevated c-erbB-2 levels died within 2 years, while 34% of the c-erbB-2-negative patients were still alive at the end of follow-up (> 3 years).

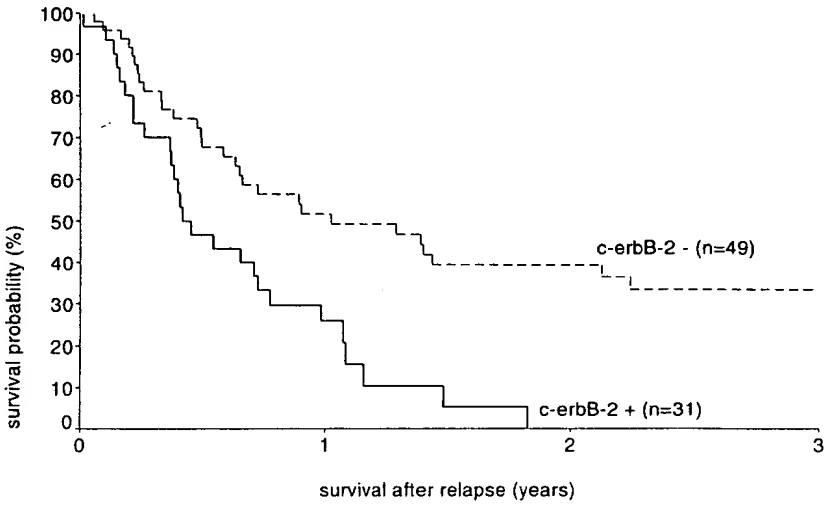

Fig. 2. Survival after relapse according to c-erbB-2 serum positivity in 80 metastatic breast cancer patients: —— c-erbB-2 positive, --- c-erbB-2 negative.

Table 2. Response to first-line therapy (hormone = hormonal therapy, chemo = chemotherapy) according to c-erbB-2 serum positivity

Response to first-line therapy	C-erbB-2-positive (n = 31)			C-erbB-2-negative (n = 49)		
	total (%)	hormone	chemo	total (%)	hormone	chemo
Complete remission	0	0	0	6 (12)	4	2
Partial remission	1 (3)	0	1	6 (12)	4	2
No change	8 (26)	2	6	17 (35)	5	12
Progressive disease	22 (71)	5	17	20 (41)	3	17
Total	31	7	24	49	16	33

Menopausal status, steroid receptor status, site of recurrence as well as relapse-free interval all had an impact on predicting clinical outcome (table 3). On the contrary, the preoperative c-erbB-2 level at the time of primary surgery, adjuvant chemotherapy regimen, initial tumor size and degree of nodal involvement had no influence on predicting survival after relapse in these metastatic patients (data not shown).

In order to evaluate the independent prognostic value of c-erbB-2 and the interrelationship of the other prognostic factors, a multivariate analysis was performed (table 3). c-erbB-2 turned out to be the strongest factor in predicting prognosis, followed by site of metastases and relapse-free interval. Estrogen receptor status lost its significance when the other factors were taken into account.

Discussion

In our study, elevated c-erbB-2 levels were found in 31 of 80 patients (38.8%) with metastatic disease. Positive rates reported in other studies have ranged from 19 to 51%. These discrepancies may be due to the differences in the cutoff levels chosen by the different authors (table 4).

Patients with elevated c-erbB-2 serum levels were less likely to benefit from treatment unrelated to type of first-line therapy (chemotherapy or endocrine therapy). While 71% of c-erbB-2-positive patients had progressive disease, only 41% of the c-erbB-2-negative patients did not respond to treatment. Similar results were obtained by Leitzel et al. [16], who investigated the influence of c-erbB-2 serum positivity on response to second-line endocrine therapy in 300 metastic breast cancer patients with positive or unknown hormone receptor status. The clinical response rate was 21% (12/58) in c-erbB-2-positive pa-

Table 3. Univariate and multivariate analysis of survival after relapse

Variable	Univariate analysis p value	Multivariate analysis p value	OR (95% CI)
Menopausal status Pre vs. post	<0.05	<0.05	1.9 (1.0–3.4)
ER status Negative vs. positive	<0.05	n.s.	-
PR status Negative vs. positive	<0.01	<0.01	1.9 (1.1–3.4)
Relapse-free interval ≤ 1 year vs. > 1 year	<0.01	<0.01	2.2 (1.2–3.9)
Site of metastases Visceral site vs. bone	<0.01	<0.01	2.7 (1.3–5.3)
C-erbB-2 Positive vs. negative	<0.01	<0.01	3.1 (1.6–5.9)

Post = Postmenopausal; pre = premenopausal; n.s. = not significant.

tients compared to 41% (99/242) in patients with nonelevated c-erbB-2 serum levels. These data are in keeping with immunohistochemical investigations demonstrating that tumors overexpressing c-erbB-2 protein were less responsive to hormonal therapy or chemotherapy [17–19].

In metastatic breast cancer, site of recurrence and relapse-free interval are considered as the main criteria to predict survival after relapse. In our study, c-erbB-2 turned out to be the strongest independent prognostic factor for predicting survival after recurrence. Metastatic breast cancer patients with elevated c-erbB-2 levels had a significantly shorter survival compared to those with normal values. Relapse-free survival, site of metastases, progesterone receptor status and menopausal status also had a significant impact on prognosis, but serum c-erbB-2 was the most powerful of these independent prognostic factors in predicting survival after relapse.

Fifteen percent of the metastatic patients had elevated c-erbB-2 levels at the time of primary surgery. In the prior case-controlled study, only 7% of the patients without recurrence were c-erbB-2-positive. The higher positive rate of serum c-erbB-2 in the metastatic group demonstrated that elevated c-erbB-2 levels in primary breast cancer were associated with an increased risk to develop metastases.

Table 4. Positive rates of c-erbB-2 in breast cancer patients with distant metastases

Author	Cutoff level	Patients	Positivity rate, %
Molina et al. [13]	15 U/ml	157	49
Narita et al. [14]	20 U/ml	51	51
Isola et al. [12]	20 U/ml	78	40
Our study	(25 U/ml) 120 fmol/ml	80	39
Leitzel et al. [16]	30 U/ml	300	19

A conversion from normal to elevated c-erbB-2 could also be detected in this recurrent patient cohort. Thirty-four percent of the c-erbB-2-negative patients were detected with elevated c-erbB-2 levels at the time of detection of metastases. Five of the 12 c-erbB-2-positive patients had a decrease to normal c-erbB-2 values when metastases were detected. These data indicate that positive c-erbB-2 concentrations at the time of detection of metastases are not associated with c-erbB-2 serum positivity at the time of primary surgery. Moreover, in this study, serum c-erbB-2 levels at primary surgery are not useful in predicting survival after relapse in metastatic breast cancer. Therefore, c-erbB-2 serum levels should be measured again at the time of detection of metastases, as changes in c-erbB-2 serum expression can occur and may be due to a change in tumor growth behavior associated with a changed prognosis.

In conclusion, our results suggest that positive c-erbB-2 levels at the time of diagnosis of metastases identify a subgroup of metastatic breast cancer patients with a poor response rate to first-line treatment and a shortened survival after relapse.

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